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REVIEW ARTICLE

Gene Editing Technology for Pharmaceutical Application

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ABSTRACT
Zinc Finger Nucleases (ZFNs), TALENs, and CRISPR-Cas9 have been discussed in this paper's thorough examination of gene-editing technologies. Scientists are able to specifically alter an organism's DNA because of to these technologies, which have transformed genetic engineering and produced significant developments in industrial biotechnology, clinical treatments, and biological research. Their history, mechanisms, and applications are also discussed in the report in detail with specific reference to their microbiological engineering, cancer therapy, disease modelling, and agricultural enhancement applications. How simple, efficient, and affordable CRISPR-Cas9 is, makes it unique and has accelerated its adoption in clinical trials and research. Also addressed in the report are the molecular mechanisms of gene editing, including DNA reparation progressions such by means of homology-directed repair (HDR) and nonhomologous end joining (NHEJ).
Keywords*: CRISPR-Cas9, TALENs (Transcription Activator-Like Effector Nucleases), ZFNs (Zinc Finger Nucleases), Mega nucleases, Cas9 protein, Guide RNA (gRNA).*

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INTRODUCTION

Many scientists and researchers have been prompted to alter genes to match desired genetic sequences by the quick development of gene-editing technologies. These days, researchers are actively working to modify the genes of a wide variety of organisms. TALEN and CRISPRCas9 are two of the most widely used and reliable gene-editing methods. CRISPR-Cas9, or "Clustered Regularly Interspaced Short Palindromic Repeats," operates by connecting these sequences to particular proteins. because of to these cutting-edge tools, scientists are able to generate unique genetic sequences that may be used to treat a number of diseases, especially cancer.[1] This particular type of gene modification, which is frequently called "nuclei engineering," involves cutting DNA at specific locations with specialized enzymes. Once the DNA has been cut, it can be modified and inserted into a target gene. Since the 1953 discovery of the DNA double helix, our understanding of genetic processes like transcription, translation, genetic coding, and epigenetic modifications has grown significantly because of to new experimental techniques. Among the nucleases that have been discovered recently are zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and more recently, CRISPR systems [2]. Of these, CRISPR has become an important component in the advancement of biological research. Numerous new opportunities have been created made possible by CRISPR, which was named Science's Breakthrough of the Year in 2015. These days, gene editing holds great promise for gene therapy and other medical uses.[3] The adaptability and precision of CRISPR-Cas9 and TALENs have revolutionized genetics and accelerated progress in domains such as human gene therapy, drug development, disease modeling, neuroscience, synthetic biology, and agriculture. Scientists can use the natural DNA repair processes that are triggered by these breaks to make precise genetic changes. Nonhomologous end joining (NHEJ), a repair technique that introduces random insertions or deletions (indels) to knock out genes, is the most widely used method. As an alternative, homology-directed repair (HDR) can be used for more accurate edits, like fixing mutations or adding new genes, if a matching DNA template is available[4]. Researchers can now change nearly any gene in a variety

of cell types and species due to the development of programmable nucleases, which has greatly accelerated the gene editing process. hemophilia, muscular dystrophy, immune deficiencies, viral infections, cardiovascular diseases, digestive disorders, and cancer immunotherapies involving T cells are among the conditions for which gene editing is currently being investigated in preclinical research. A few of these applications are currently advancing to clinical trials in phases I and II [5]. This review examines at the most popular gene editing methods, TALENS, CRISPR/Cas9, and ZFNs, with a special focus on eukaryotic cells and animal models. It explores how these devices and their modified forms could be used to treat a range of human diseases in besides highlighting current clinical studies and the challenges in bringing gene editing therapies to the clinic [6].

The purpose and goal

Purpose

To conduct studies and assess the potential of creating new pharmaceutical applications in drug discovery and disease treatment using gene editing technologies such as CRISPR-Cas [7].

Goal

- To understand the principles and mechanisms behind gene editing technologies associated with pharmaceutical research, such as CRISPR-Cas9, TALENs, and ZFNs.[8]
- To examine the potential therapeutic applications of gene editing for the treatment of genetic diseases such as cystic fibrosis, sickle cell anemia, and muscular dystrophy.[9]
- To evaluate the application of gene editing in drug target validation and development using in vitro and in vivo models. [10]
- To analyze gene editing's safety, specificity, and ethical concerns in pharmaceutical applications.[11]
- To examine recent developments and case studies in which gene editing has resulted in effective clinical trials or pharmaceutical interventions.[12]

Context

The main purpose of programmable nucleases that produce targeted DNA double-strand breaks (DSBs) is to precisely alter genetic material. The cell's natural DNA repair pathways are triggered by these breaks and have the ability to add, remove, or alter DNA at particular sites. Even more accuracy and efficiency can be achieved with more sophisticated instruments, such as nickases, which only cut one strand of DNA. Furthermore, genes can be altered by synthetic molecules called peptide nucleic acids (PNAs), which resemble DNA but do not actually cut the DNA. Nonhomologous end joining (NHEJ) and homology-directed repair (HDR) are the two primary methods for fixing DSBs [13]. repairing mutations is its primary goal. NHEJ can be useful for gene knockout even though it is less accurate because it directly joins broken DNA ends and frequently introduces tiny insertions or deletions (indels). Nonhomologous end joining (NHEJ) and homology-directed repair (HDR) are the two main approaches for fixing DSBs. Its main objective is to fix mutations. NHEJ is useful for knocking out genes despite its lower accuracy because it directly rejoins the broken ends of DNA and frequently introduces tiny insertions or deletions (indels). Two DSBs on the same chromosome can have their sequences eliminated or changed [14]. Chromosome translocations may happen if they are occurring on distinct chromosomes. the development of numerous vaccines and therapies for chronic illnesses may be aided by this technology. researchers think that gene editing technologies could help millions of people with diseases like HIV, cancer, and hemophilia by managing or even curing them. Scientists have been able to introduce new genetic material into living things since the 1970s thanks to genetic engineering [15]. One drawback, though, has been the DNA's haphazard integration into the host genome, which may inadvertently interfere with other crucial genes. More accurate targeting of the inserted genes to particular locations in the genome is now possible thanks to new techniques that have surfaced in recent years. These developments have made it possible to precisely edit specific DNA sequences and decreased off-target effects. These developments have made it possible to precisely edit specific DNA sequences and decreased off-target effects. These advancements can be used in scientific research, gene therapy, and the repair of damaging genetic mutations. Treating some genetic disorders may involve replacing a defective gene with a healthy one [16].

Types of gene editing

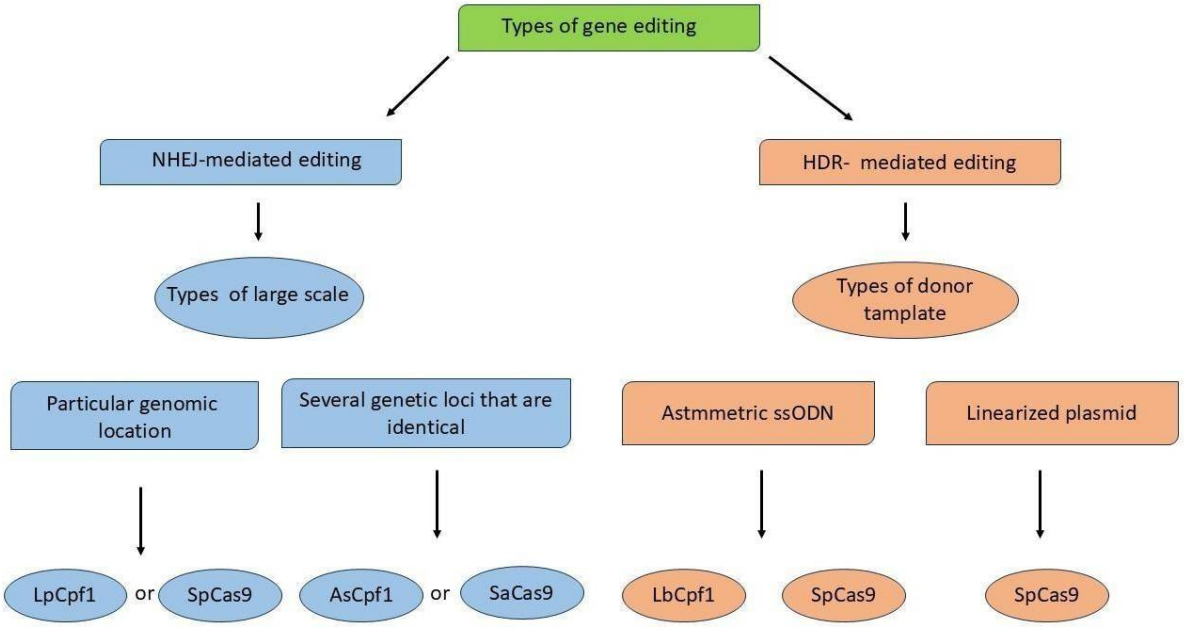

Fig1: types of gene editing technology

Purpose of gene editing

Scientists can alter an organism's DNA using a set of tools called genome editing, sometimes referred to as gene editing. These methods make it possible to precisely add, remove, or modify genetic material at specific locations throughout the genome. CRISPR-Cas9 is one of the most well-known genome editing techniques that have been developed over time. in comparison to previous methods, CRISPR, which stands for "clustered regularly interspaced short palindromic repeats," and its related protein Cas9 have garnered a lot of interest from the scientific community because of their accuracy, speed, affordability, and efficiency [17]. It functions by generating guide RNA, a brief RNA sequence that points the Cas9 enzyme in the direction of a particular spot in the DNA. Although Cas9 is the most widely used, Cpf1 and other enzymes can also carry out comparable tasks. Once the DNA has been cut, researchers can replace it with desired DNA segments, add new sequences, or remove sections using the cell's built-in repair mechanisms. A promising method for diagnosing and treating human illnesses is genome editing [18]. It is frequently used in labs to investigate disease processes in animal models and cells, and studies are still being conducted to assess its efficacy and safety in people. In addition to more complex diseases like HIV, cancer, cardiovascular disorders, and neurological conditions, genome editing may be used to treat inherited conditions like hemophilia, sickle cell anemia, and cystic fibrosis.[19] Despite the fact that DNA editing tools have been around for decades, contemporary methods have greatly increased genome editing's accuracy, speed, and affordability. These developments expand on cells' innate capacity to mend broken DNA. Examples of engineered protein-based editing systems include transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and mega nucleases. Among them, CRISPR/Cas9 is a particularly popular and effective technique [20].

Application

Scientists can add, alter, or remove almost any DNA sequence in a broad range of cells and organisms through genome editing. Although methods for changing DNA have existed for many years, new developments have accelerated, reduced the cost, and improved the efficiency of the procedure. This method is founded on the finding that a gene's DNA breaks and the cell's natural repair mechanism fixes it. To make specific changes, genome editing imitates this natural repair process [21]. Mega nucleases, zinc-finger nucleases, and transcription activator- like effector nucleases (TALENs) are a few of the more sophisticated protein-based genome editing tools. CRISPR/Cas9, or clustered regularly interspaced short palindromic repeats, is another popular method. Nearly every human disease, including viral infections, cancer, blood disorders, immunological deficiencies, metabolic diseases, muscle disorders, congenital lung diseases, neurological problems, skin disorders, inherited eye conditions, and genetic hearing loss, is being treated with genome editing. Clinical Trials.gov currently lists more than 80 clinical trials, most of which focus on different types of cancer, type 1 diabetes mellitus (T1D), some viral diseases, and a number of single-gene disorders [22].

Table 1: application of gene editing technology

Technique Name	Description	Advantages	Applications
CRISPR-Cas9	Uses gRNA and Cas9 to make a double-strand break at specific sites	Simple, cost-effective, precise	CAR-T cell therapy, sickle cell treatment
Zinc Finger Nucleases (ZFNs)	Engineered proteins cut DNA at specific locations using FokI	High specificity	HIV therapy, hemophilia treatment
TALENs	TALE proteins bind specific bases; FokI cuts the DNA	High accuracy	Sickle cell treatment, stem cell editing
Nickase CRISPR	Modified Cas9 cuts only one DNA strand	Reduced off-target effects	Safer precision editing
Base Editing	Chemically changes one base without cutting both strands	No double-strand breaks	Point mutation correction
Prime Editing	Cas9 nickase + reverse transcriptase edits without breaks	Versatile, accurate	Tay-Sachs disease correction
HDR (Homology-Directed Repair)	Uses donor template for accurate DNA repair	High fidelity	Cystic fibrosis, gene correction
NHEJ (Non-Homologous End Joining)	Repairs breaks without a template, causing indels	Fast, no donor required	Gene knockouts, cancer models

a. Genetic manipulation and bioengineering

Until the development of engineered nucleases, altering mammalian cell lines was an expensive and time-consuming procedure that was usually reserved for specialized labs with highly skilled personnel. With the advent of affordable and easy-to-use gene-editing tools, it is now possible to create customized cell lines with almost any genetic alteration in a matter of weeks. Procedures like gene knockout, deletion, inversion, correction, insertion, and even chromosomal translocation have become much more commonplace thanks to these tools [23]. In addition to altering cell lines, targeted nucleases have sped up the production of genetically
modified organisms, enabling the quick development of transgenic livestock, mice, rats, zebrafish, and monkeys. When combined, these developments greatly improve the capacity to simulate human illnesses and aid in the creation of novel treatments [24].

b. Cancer Research and Genome Editing

In cancer research, oncogenes and mutant tumor suppressor genes have emerged as key targets for genome-modifying techniques. The development of gene editing technology has resulted in notable advancements in targeted gene modification, starting with its initial demonstrations in eukaryotic cells and continuing with more recent developments in engineering hematopoietic stem cells (HSCs) and tumor-targeted T cells. Additionally, these discoveries have broadened the focus of cancer research [25]. Zinc Finger Nuclease (ZFN)-mediated targeting is a crucial method in this field that has been crucial for genome editing. Researchers are now able to precisely alter the genome thanks to ZFNs, a groundbreaking platform for programmable DNA cleavage. For instance, the telomeric region of the breakpoint cluster region of the mixed lineage leukaemia (MLL) gene was targeted by a specially created ZFN. This was carried out in order to investigate chromosomal rearrangements linked to MLL leukemogenesis through DNA double-strand break (DSB) repair[26]. A mouse cell line obtained from patients with chronic myeloid leukaemia (CML) was used to test this strategy. Furthermore, ZFNs were designed to interfere with the β- and α-chain genes of the endogenous T cell receptor (TCR), leading to customized control and altered T cells that did not express CD3-TCR on their surface and stimulated growth by elevating interleukin-7 (IL-7) and IL-15 levels [27].

C. Liver-Targeted Gene Editing

Apart from ex vivo gene editing of blood and immune cells, in vivo gene editing is gaining popularity for targeted gene insertion and correction in organs where cell transplantation is difficult or not practical. This calls for the efficient delivery of donor vectors and gene-editing tools to particular tissues. Zinc Finger Nucleases (ZFNs) and a factor IX cDNA (without a promoter) were introduced into the liver of a mouse model of hemophilia B using AAV vectors in one of the earliest successful in vivo demonstrations of nuclease-mediated gene editing. The hemophilic phenotype was corrected as a result of this experiment [28]. Subsequent studies in adult mice, whose hepatocytes were most likely not in the cell cycle,

demonstrated comparable effectiveness. Nevertheless, both HDR and non-homologous end joining (NHEJ) repair mechanisms continued to drive gene integration. To fully comprehend the function of the cell cycle and the effectiveness of gene editing with AAV and other delivery vectors in diverse tissue types, more investigation is necessary. Numerous genetic disorders, such as lysosomal storage diseases like Fabry disease, Gaucher disease, Pompe disease, von Gierke disease, Hurler and Hunter syndromes, and clotting disorders like hemophilia A and hemophilia B, may be treated by targeted gene correction in the liver [29].

d. The use of gene editing in scientific studies

Numerous organisms have had their genomes edited for scientific purposes, especially using CRISPR technology. By developing "knockout" models in various species, it allows scientists to investigate the genetic origins of illnesses. This method makes it possible to comprehend the genetic foundation of particular conditions better. Pig organs may one day be used as a source for human organ transplants since CRISPR is also being used to deactivate viruses in pigs. Furthermore, CRISPR makes it possible to alter genes in particular tissues or organs, which improves the efficiency of disease research by concentrating on the genes that cause the illness [30]. Additionally, it is used to develop disease models in cells, including those made from human pluripotent stem cells. gene drive technology is one of the most promising uses of CRISPR. It significantly accelerates the spread of genetic alterations in populations of sexually reproducing animals, outpacing the rate of natural evolution. By altering mosquito populations, this could lessen the spread of infectious diseases [31].

Various approaches of gene editing

Gene editing technology is linked to a variety of techniques, and scientists and researchers can develop new techniques using this process. The techniques that are frequently used are (1). The two main parts of genome editing tools are one that can precisely manipulate nucleic acids, similar to a microscalpel, and another that can quickly navigate through billions of bases to find the desired nucleic acid sequence. The three most widely used genome editing technologies CRISPR/Cas-based RNA-guided DNA endonucleases (CRISPR/Cas), Zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs)-have different methods for editing genomes. With the creation of engineered nucleases like ZFNs, TALENs, and CRISPR, significant advancements in human genome editing have been made [32]. The most recent of these, CRISPR/Cas, is notable for its high editing efficiency and user-friendliness. Restoring the gene's normal expression and function is the main objective of gene therapy, as opposed to gene replacement. The newest tool in the genome-editing toolbox, the CRISPR- Cas9 system, is crucial for bacterial immunity. It aids bacteria in protecting their DNA from plasmids and viruses. Cas proteins are guided by RNA to cleave particular DNA sequences in order for the system to function. The CRISPR region of the bacterial genome receives brief

segments of foreign DNA from viruses or plasmids, which are then transformed into CRISPR RNA (crRNA). Together with tracrRNA, the crRNA directs the Cas9 protein to eliminate the invasive DNA in a particular order [33]. By eliminating the tracrRNA and merging it with the crRNA to form a single guide RNA (gRNA), researchers have streamlined this system for genome editing over time. Because the gRNA can identify and direct the Cas9 protein to the proper location, engineers do not need to create new proteins for each new target site. It is now among the quickest methods available to scientists for introducing new genes into the genome of an organism. The CRISPR genes in bacteria, which aid in their ability to identify and combat viruses, served as the inspiration for the technology. This system is especially helpful for researching the behaviour of foreign DNA inside cells, especially in laboratory settings. The Cas9 nuclease, which is directed by a tracrRNA molecule acting as a "pilot," cuts double- stranded DNA at a specific location as part of the CRISPR-Cas9 system [34].

CRISPR-Cas9 can be used in genome editing in three primary ways

Plasmid-based CRISPR/Cas9:

In this technique, the Cas9 protein and sgRNA are made in the lab by assembling a plasmid into a single molecule. It offers longer-lasting Cas9 and sgRNA expression and prevents the need for repeated transfections. Getting the plasmid into the nuclei of the target cells is a major obstacle, though [35].

1. Direct Cas9 mRNA and sgRNA delivery: This approach is easier to use, but it has the drawback of low mRNA stability, which results in transient gene editing and short-term expression.
2. Direct delivery of sgRNA and Cas9 protein: This method is a desirable choice for genome editing because it is quick, stable, and less likely to cause an immunological reaction [36].

CRISPR-Cas System Classification and Interference Mechanism

Based on the structure of the Cas proteins and effector complexes they employ, the six types of the CRISPR-Cas system (Types I through VI) currently fall into two main classes, Class 1 and Class 2. Each of the 33 subtypes and various variations is identified by a distinct cas gene. To mount an immune defense, Class 1 systems which include Types I, III, and IV rely on a complex composed of several Cas protein subunits.

However, to defend against invaders, Class 2 systems which include Types II, V, and VI use only one Cas protein effector. Almost all CRISPR-Cas systems (except Type III) need to recognize a brief DNA sequence adjacent to the target, known as the protospacer adjacent motif or PAM, in order to perform their two primary roles of interference and adaptation [37]. A collection of proteins called the CRISPR associated complex for antiviral defense (Cascade) recognizes and cuts target DNA in Class 1 systems, especially Type I. One Cas5, one Cas6, several Cas7 proteins, and a signature protein known as Cas3 are usually included in this complex. The Cas6 family proteins process the initial crRNA transcripts (pre-crRNAs) in the majority of Type I systems, with the exception of the I- C subtype that employs Cas5d. The Cse1 (also called Cas8) subunit leads the Cascade complex in its initial scan of the target DNA for a PAM sequence when the Type I system is active. Cascade creates an R-loop structure to aid in locking onto the DNA after determining the correct PAM. The complex then changes shape and attracts the Cas3 protein to start cleaving the target DNA via the Cse1 subunit. Utilizing Gene Editing Based on CRISPR in Microbial Engineering Food production, medicine, agriculture, and manufacturing are just a few of the industries that have long relied on microorganisms [38]. They are essential to contemporary industrial biotechnology because of their growing significance to both industry and human life. Researchers are now doing more than just separating microorganisms from their natural sources thanks to developments in biological technology. Rather, they are creating enhanced microbial strains artificially through the use of gene editing tools. These improved strains are essential for raising the yield and caliber of industrial fermentation-produced goods. Effective breeding methods are required to get the most out of microbial resources. One of the most notable advances in genetics, CRISPR-based gene editing, has significantly increased the efficiency of contemporary microbial strain development. This section focuses on the genetic modification of probiotics and the application of CRISPR-Cas gene editing technology to industrial microbes [39].

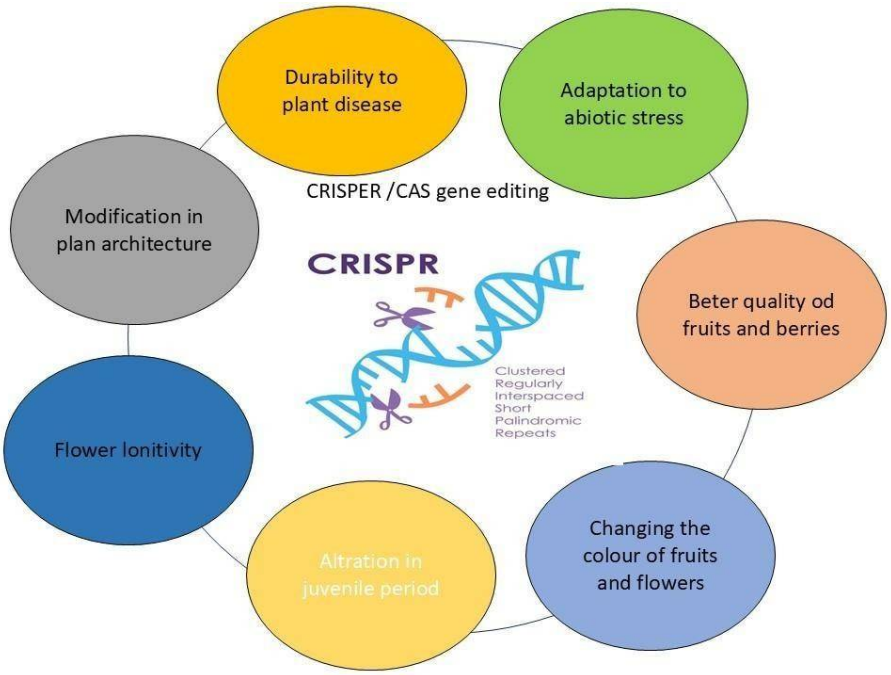

Fig 2: CRISPER/CAS gene editing approaches

Utilizing industrial microorganisms

For bio industrial production, high-quality microbial "chassis" cells are essential. These industrial strains can now be precisely enhanced thanks to gene editing technologies like CRISPR-Cas. Researchers can create highly effective "cell factories" to create better and more varied products by optimizing microbial metabolism, enhancing component compatibility, and creating artificial metabolic networks [40]. Through genetic engineering, microorganisms can create novel or difficult-to-make compounds, making industrial processes more economical and cleaner. Escherichia coli, Bacillus subtilis, Corynebacterium glutamicum, and Saccharomyces cerevisiae are examples of frequently used microbial chassis [41]. E. coli is a popular choice among these because of its quick growth, versatility, and ease of handling. The ability of CRISPR/Cas9 to target the genome of E. coli was first demonstrated in 2013, resulting in a 65% mutation rate and allowing mutations with the aid of λ-Red proteins. Since then, the industrial use of E. coli has greatly

improved thanks to CRISPR-Cas9. For instance, Li et al. achieved almost 100% editing efficiency when they created a CRISPR-based technique for metabolic and repeated genome editing [42].

1. Delivery of CRISPR/Cas9

In order to successfully employ CRISPR/Cas9 for gene editing, it must be introduced into the target cells or tissues using appropriate delivery techniques or vectors. Recombinant viral vectors have been developed to give infected cells or tissues beneficial traits by taking advantage of viruses' capacity to transfer exogenous genetic material cells [43]. Adenoviruses, lentiviruses, and adeno-associated viruses (AAV) are frequently employed viral vectors in genome editing treatments and clinical research. Another worry is that certain viral vectors have the ability to permanently incorporate the altered DNA into the host genome, which increases the possibility of chromosomal abnormalities or unexpected changes [44].

2. Adenovirus

Adenoviruses are between 80 and 100 nm in size and contain double-stranded DNA. Its genome is between 34 and 43 kb long and can accommodate about 8 kb of foreign DNA. By adding more targeting signals, the effectiveness of adenovirus vector-mediated CRISPR/Cas9 delivery can be improved, utilizing the vector's potent ability to transport substantial genetic loads. Certain adenoviral vectors can now transport up to 37 kilobases of target DNA by lowering their own genetic content. Both dividing and non-dividing cells can be infected by these vectors [45]. One problem still exists, though: their genomes do not synchronize or integrate with the genome of the host cell, which actually lessens undesirable mutations and off-target effects. Even though viral vectors may increase their capacity to kill tumor cells by inciting an immune response, future vector delivery is unaffected by the immune response, especially the B cell response. However, reducing the immunological reaction that these vectors elicit would greatly increase their efficacy and safety in gene transfer [46].

3. AAV

AAVs are composed of a 4.7 kb ssDNA genome and an icosahedral protein capsid that is roughly 26 nm wide. Because of their intriguing properties, such as the need for virulence (the capacity to multiply in other cells), long-term gene expression, and the capacity to survive in both dividing and non-dividing cells, AAV vectors are widely used in in vivo transfection systems. Features of AAV vectors in the growth of the AAV family include varied tropism targeting different organs and serotype phenotyping [47]. AAVs are excellent delivery vehicles, but they have limitations when it comes to in vivo CRISPR/Cas9 delivery. The ideal AAV transmission capacity is estimated to be between 4.1 and 4.9 kb. Since the SpCas9 protein, for example, is about 4.2 kb in size and recombinant AAV must also contain essential regulatory components for gene expression, AAVs cannot be used to provide broad gene regulation [48].

4. Zinc-Finger Nucleases

Originally developed by combining the DNA-cleaving domain of the restriction enzyme FokI with a specially made Cys2-His2 zinc-finger protein, zinc-finger nucleases (ZFNs) were the first extensively used genome editing tools. ZFNs work as dimers, with each monomer attaching to a particular DNA sequence known as a "half site," which is usually composed of nine to eighteen base pairs. The enzyme can cut DNA within a spacer region of roughly five to seven base pairs between the zinc-finger binding sites because the FokI cleavage domain brings the two ZFN monomers together. Three or four zinc-finger domains, each with about thirty amino acids arranged in a ββα structure, are typical of a ZFN [49]. First discovered in 1985 as a component of transcription factor IIIa in Xenopus oocytes, zinc-finger proteins are renowned for their ability to precisely bind DNA. By means of conserved interactions with complementary DNA sequences, the Cys2His2 zinc-finger domains establish the binding specificity. Comprising approximately 30 amino acids, each Cys2His2 zinc finger forms two antiparallel β sheets opposite an α-helix. By inserting into the major groove of the DNA and creating base-specific contacts, these structures enable the finger to recognize particular DNA sequences. The FokI type II restriction enzyme, which is responsible for the DNA-cleaving portion of the ZFN, must dimerize in order to cut the DNA at the desired location, allowing for precise genome editing [50]. ZFNs are essentially synthetic restriction enzymes that combine a nonspecific DNA-cleaving domain with a zinc finger-based DNA-binding domain. Each ZFN uses three to six zinc finger repeats to recognize a sequence of 12 to 18 base pairs. If the fingers are made to bind precisely to the desired sequence, even a set of ZFNs with three fingers that can each recognize a total of 18 base pairs can target a distinct location in the mammalian genome. Two ZFNs are required to cut DNA at non-palindromic sites because the FokI cleavage domain requires dimerization [51].

4. TALENs

The discovery of a simple one-to-one coding regulating the DNA-binding specificity of TALE proteins from the plant pathogen Xanthomonas once again raised the exciting possibility of modularly designing new DNA-binding proteins. Highly conserved 33-35 amino acid TALE repeats bind a single base pair of DNA, and their specificity is dictated by two hypervariable residues. When the protein wraps around the DNA in a

super helical structure, the hypervariable residue is presented into the main groove because each repeat creates a two-helix structure connected by a loop, according to crystal Designing modular DNA-binding proteins has gained new attention since the identification of a straightforward one-to-one code that controls the DNA-binding specificity of TALE proteins from the plant pathogen Xanthomonas [52]. According to structural studies, each repeat creates a two-helix structure joined by a loop, and the protein wraps around the DNA in a super helical pattern, with the variable residue projecting into the major groove. Researchers can make lengthy chains that precisely bind particular DNA sequences by joining several TALE repeats. TALE arrays can be assembled using a variety of techniques. high-throughput, more sophisticated methods employ ligation-independent cloning or solid-phase assembly. Scientists swiftly created TALENs by combining these domains with the DNA-cleaving portion of the FokI enzyme, which works similarly to ZFNs, after discovering that TALEs are programmable DNA-binding proteins [53]. It has been demonstrated that TALENs effectively induce both non-homologous end joining (NHEJ) and homology directed repair (HDR) in human somatic and stem cells. They function in pairs to cut target DNA. TALENs are superior to ZFNs in two key ways. First, the development of new nucleases is accelerated and made easier by the fact that TALE arrays can be created without the need for selection or intricate evolutionary processes. Second, when compared to certain ZFNs, TALENs typically have lower toxicity and higher accuracy. notwithstanding these advantages, TALENs are much bigger and have a lot of repetitive sequences, which makes it difficult to deliver them into cells, particularly when using viral vectors like AAV that have size restrictions [54]. Alternative strategies have been created to get around these obstacles, though. Recombination-resistant RVD arrays can be created by substituting different codons or amino acids, and TALENs can be delivered as mRNA or even directly as proteins. TALENs have also been successfully delivered to cells that are difficult to transfect using adenoviral vectors. The only significant requirement for TALENs to target nearly any DNA sequence is a thymine (T) at the 5' end, which is imposed by the TALE array's N-terminal region. TALENs are a powerful platform for accurate gene editing because of their wide targeting ability and relative ease of engineering. However, in vivo delivery is still hampered by their size and repetitive nature. Interestingly, a zinc finger only needs roughly 30 amino acids to bind three bases, while each TALE repeat needs 34 amino acids to recognize one base pair [55].

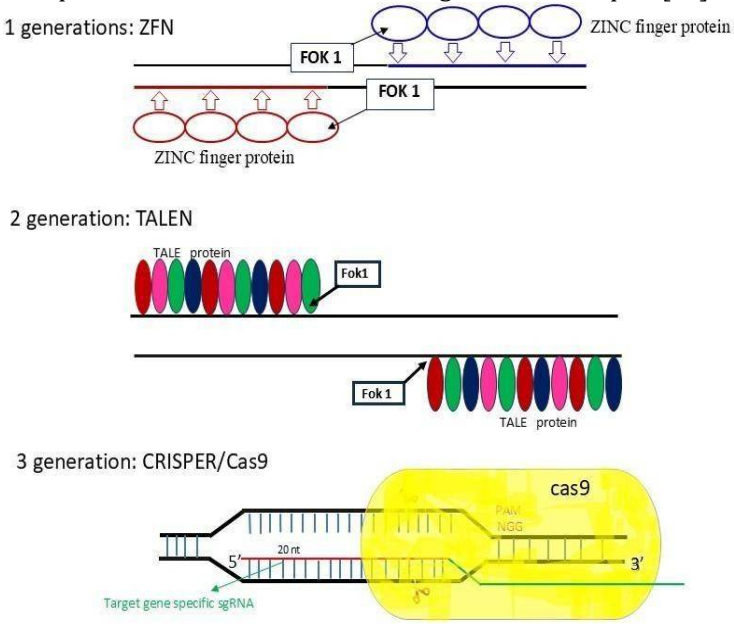

Fig 3: mechanism gene editing technology

Mechanism of Gene Editing Technology

The basis for gene editing technology was the idea that the body's natural repair mechanisms could be triggered by targeted DNA double-strand breaks (DSBs). Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are the two main techniques used to fix these DNA breaks. In order for HDR to fix the break, the damaged DNA strand must invade a homologous sequence and use it as a template [56]. The efficiency of gene targeting through homologous recombination in mammalian cells can be greatly increased by introducing a DSB at a target site, according to groundbreaking research conducted by Maria Jasin's group. Conversely, fixes DSBs by directly reassembling the cleaved DNA ends without the

need for a template. Because these indels have the ability to impair the function of the target gene, NHEJ is a useful technique for gene knockout applications in a variety of cell types and organisms [57].

1. **Gene Knockout/Mutation**

By exploiting the inherent errors in NHEJ, the most basic type of gene editing entails adding tiny indels at the target location. Alternative NHEJ, also known as microhomology-mediated end joining (MMEJ), necessitates ligation of the DNA ends, end resection, and the annealing of brief single-stranded microhomologous sequences. However, unprocessed DNA ends are directly religated by classical NHEJ. Throughout the cell cycle, both forms of NHEJ are active and often cause mutagenesis, which leaves indels at the break site. These indels frequently result in frameshift mutations when the nuclease target site is located within a gene's coding sequence [58]. The gene can be disrupted by these mutations, which is especially helpful for gene knockout. NHEJ-induced indels can aid in restoring the gene's correct reading frame in conditions like Duchenne muscular dystrophy (DMD), where gene deletions result in frameshifts and loss of protein function. One of the most popular uses of targeted mutagenesis is gene knockout, which enables the irreversible disruption of gene function. This contrasts with conventional gene therapy, which typically entails introducing foreign sequences into the genome. Targeting dominant gain-of-function mutations, like those found in Huntington's disease, is one use for gene knockout. The huntingtin (HTT) gene's repeat expansion causes this disorder by producing a toxic HTT protein. Patients with Huntington's disease may benefit therapeutically from the removal of the mutant allele through NHEJ-based gene editing [59].

2. **Gene Correction**

By integrating a donor template into the genome, targeted DSBs can also be utilized for accurate gene correction through HDR. Compared to the random mutations brought on by NHEJ, this approach is more predictable. During the S and G2 phases of the cell cycle, in particular, HDR naturally takes place using the sister chromatid as a repair template. To direct the repair, scientists can also add an exogenous donor sequence. High-efficiency HDR-based gene editing is made possible by delivering particular nucleases together with a targeting vector that contains DNA that resembles the break site [60]. Several studies have shown that by incorporating the required sequence variations from the donor template into the endogenous gene, disease- causing mutations can be fixed. Single-stranded oligonucleotides (ssODNs), which can have as few as 80 base pairs of homology, are frequently utilized as donor templates for HDR. Viral vectors like integrase-deficient lentivirus or adeno-associated virus (AAV) can also be used as sources of donor DNA for cells that are challenging to transfect. Because of their inherent recombinogenic characteristics, AAVs in particular are appealing for donor DNA delivery, particularly when employing effective hybrid serotypes like AAV-DJ [61].

3. **Insertion of Genes**

Earlier clinical trials using murine retroviral vectors showed significant risks of insertional mutagenesis, despite the fact that viral vectors have long been used in gene therapy to insert exogenous genes into the genome. This risk results from these viruses' incapacity to regulate the location of genetic material integration into the genome. However, more controlled integration is possible with site-specific DNA insertion via DSB-induced HDR, which uses a donor template with homology arms sequences that match the nuclease cut site on either side of the desired genetic insert [62]. This method can guide the insertion of therapeutic transgenes into "safe harbor" loci, which are particular genomic locations. These loci permit high levels of gene expression while lowering the risk of insertional mutagenesis. In order to maintain control over gene expression by natural regulatory elements, a wild-type copy of a disease-causing gene can also be inserted into its corresponding endogenous locus, where it will be regulated by the gene's natural promoter. treat additional conditions such as cystic fibrosis and diabetes. However, despite its significance, it poses several challenges related to the functioning of gene- editing techniques [63]. There is a lot of interest in using genome editing for both human disease prevention and treatment. Currently, research institutes are using genome editing in cells and animal models to better understand diseases. Scientists are still researching this method's safety and effectiveness for human use. Research and clinical trials are being conducted to examine a wide range of diseases, including single-gene disorders such as hemophillia, sickle cell anemia, and cystic fibrosis. It may also be used to prevent and treat other illnesses, such as mental disorders, cancer, heart disease, and HIV infection [64].

Advantages

Thanks to biotechnology, science and technology have progressed to the point where they have established several techniques that can help researchers create new objects and experiments. Using the techniques required to modify the genome of Thanks to the living thing, numerous researchers have been able to develop different approaches in the domains of environmental science, biomedical science, disease management, and agricultural science. Haematology, cancer, organ transplantation, dermatology,

neurology, and hepatology are just a few of the diseases for which gene editing tools and technologies have been applied to help develop preventative measures and treatment strategies. The potential of this technology to offer innovative treatment approaches for chronic and life-threatening illnesses is being studied by a wide range of scientific and technological disciplines[65]. Ethical issues arise when human genomes are altered using genome editing technologies such as CRISPR-Cas9, particularly since the majority of the alterations are limited to somatic cells those that are not egg or sperm cells (germline cells). These changes only occur in particular tissues and are not inherited by subsequent generations. On the other hand, changes made to the genes in an embryo or in sperm or egg cells may be passed down to future generations. CRISPR/Cas9 The appropriateness of using this technology to improve common human characteristics, like height or intelligence, is a significant ethical question surrounding the editing of germline cells and embryos. The United States and many other countries currently prohibit germline and embryo genome editing due to ethical and safety concerns [66].

CONCLUSION

A groundbreaking development in contemporary biotechnology, gene editing has the potential to fundamentally alter medical, agricultural, and industrial procedures. Researchers can now edit the genome with previously unheard-of efficiency and precision thanks to the development of precision tools like CRISPR-Cas9, TALENs, and ZFNs. In addition to speeding up the development of disease models and treatment approaches, these technologies have created new opportunities in the fields of microbial engineering, cancer treatment, and genetic disorder repair. In particular, CRISPR's ease of use, affordability, and adaptability have contributed to its widespread acceptance. Despite these astounding advancements, there are still significant safety, ethical, and legal concerns regarding gene editing, particularly in relation to germline modifications. The potential for heredity in germline modifications necessitates careful ethical consideration, even though somatic cell editing offers promising therapeutic benefits that do not affect future generations. To put it briefly, gene editing holds enormous promise for improving biological research and preventing genetic diseases. To ensure that its advantages are realized without sacrificing human rights or societal values, it will be crucial to use it appropriately, guided by stringent moral principles and extensive legal protections.

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